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Evaluating liposomal nanoparticles for controlled release of chemotherapeutics *in vitro* and *in vivo.*

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The clinical use of chemotherapeutic drugs is greatly hampered by a combination of severe side effects on healthy organs and low accumulation in the tumor tissue. Although entrapment in stable liposomes has long been known to increase tumor accumulation while protecting the drug in circulation, drug release after accumulation is generally low, leading to poor bioavailability and consequently poor therapeutic effect [1,2]. This essentially underlines the paradoxical problem of how to simultaneously maintain liposome stability in circulation and obtain efficient drug release at the tumor site.

One compelling solution is utilizing an endogenous trigger mechanism that relies on a difference between microenvironment of the tumor and the healthy tissue. Secretory phospholipase A_2 ($sPLA_2$) is reported to be expressed at an elevated level in many tumor types [3,4]. This enzyme catalyzes the hydrolysis of phospholipids, producing equimolar concentrations of lysolipids and free fatty acids [5]. For liposomes this has a dual effect: rupture of the membrane, causing site specific release of encapsulated drug, and production of potentially lytic agents that can permeabilize the cell membrane mediating more efficient drug uptake [6,7] *(Figure 1)*.

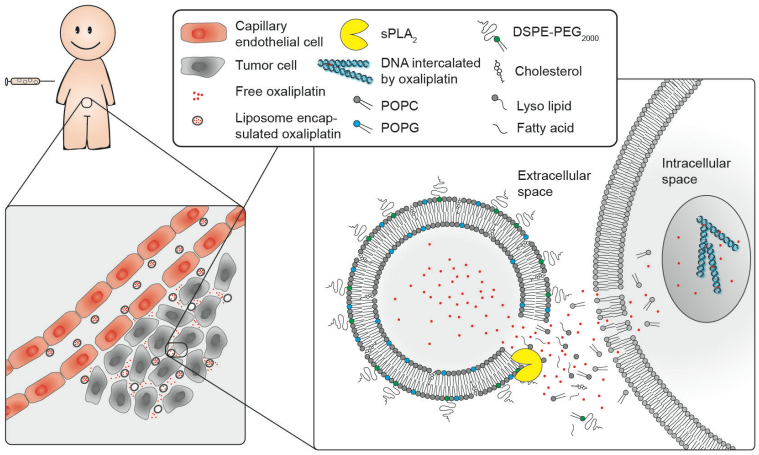

Figure 1: Conceptual illustration. Liposome encapsulated oxaliplatin will circulate until it encounters the fenestrated capillaries in the tumor tissue, where it extravasates. Here it encounters an elevated level of secretory phospholipase A_2 ($sPLA_2$), which hydrolyses the phosphoglycerolipids, causing release of the drug. In addition the hydrolysis products, lyso-lipids and free fatty acids, may act as permeability enhancers, thus further contributing to drug transport across the cellular membrane.

The concept of $sPLA_2$ responsive liposomes has been widely studied in cell free systems and in vitro [7–10]. Yet very few studies have reported on in vivo data [11,12], and only one formulation with this concept has made it to clinical trials [13]. Here we present the

rational design of liposomes optimized for secretory phospholipase A_2 ($sPLA_2$) triggered drug release, and test their utility *in vitro* and *in vivo*.

Studies with MALDI-TOF MS revealed an $sPLA_2$ dependent hydrolysis of liposomal phospholipids, demonstrating that these nanoparticles are truly enzyme sensitive. Further, *in vitro* release studies with ICP-MS disclosed enzyme dependent release of the liposome encapsulated drug oxaliplatin, signifying their potential for controlled release of cancer drugs.

Treatment of two different cancer cell lines with liposomal oxaliplatin showed efficient growth inhibition compared to that of clinically used stealth liposomes. In the presence of excess $sPLA_2$ the liposomal oxaliplatin was also superior to free oxaliplatin, suggesting a boosting therapeutic effect by the lysis products, possibly due to enhanced cellular uptake over a slightly permeabilized membrane. Empty liposomes induced a small $sPLA_2$ dependent growth inhibition, but did not demonstrate a severe cell death profile, implying that these liposomes should be inactive, and thus safe, in circulation, where the $sPLA_2$ level is low.

Although the *in vitro* results were promising, real clinical potential can only be disclosed by *in vivo* evaluation. For this purpose we utilized the human, $sPLA_2$ secreting, mammary carcinoma cell line MT-3, transplanted onto female nude NMRI mice. Mice received 10 mg/kg oxaliplatin, the liposomal equivalent or isotonic glucose solution by tail vein injection. Three days after the first treatment all mice having received liposomal oxaliplatin were euthanized due to severe systemic toxicity (excessive weight loss, dehydration and subcutaneous bleedings). Mice having received control compounds showed no signs of discomfort.

Although speculated to be related to the phospholipid hydrolysis products, the exact mechanistic cause of the systemic toxicity is not yet known. Preliminary histopathology studies of liver sections displayed acute multifocal necrosis of hepatocytes with a collapse of hepatic sinusoids and hydropical injury to the cell nuclei, which is believed to be the biological cause of the observed toxicity. Consequently, the *in vivo* study was not repeated.

The present study demonstrates that great caution should be implemented when utilizing $sPLA_2$ sensitive liposomes. Even though many have shown potential *in vitro*, the real utility can only be disclosed *in vivo*.

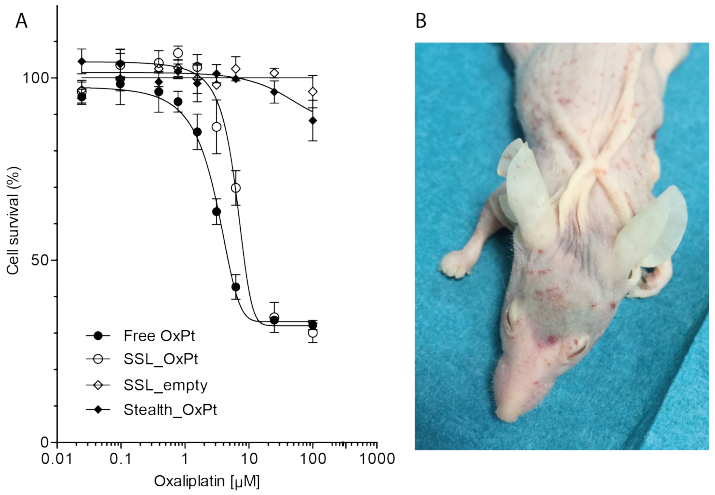

Figure 2: Effect of $sPLA_2$ sensitive liposomes. A) *In vitro* antiproliferative effect of free oxaliplatin (OxPt) (closed circles), empty (open diamonds) or OxPt loaded (open circles) $sPLA_2$ sensitive liposomes (SSLs) or OxPt loaded Stealth liposomes (closed diamonds). Cell survival was evaluated by MTS staining. Values are mean of triplicates ± SD. All values are normalized to non-treated cells. The data is representative of minimum three separate experiments. B) *In vivo* evaluation. Mouse treated with oxaliplatin loaded $sPLA_2$ sensitive liposomes was euthanized 3 days after first treatment due to excessive weight loss, dehydration and subcutaneous bleedings.

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